Claims

1. A method for detection and identification of principles from extracts of plants or animal, natural or synthetic sources, using chromatographic finger printing techniques, said method comprising the steps of:
   i) extracting the organic or organo-metallic molecules using a suitable solvent;
   ii) subjecting the extract obtained in step (i) to the separation analysis, using High Pressure Liquid Chromatography techniques;
   iii. generating contour and 3D chromatograms of the ingredients eluted based on the pH and polarity;
   iv. converting the 3-D and contour chromatogram obtained into a colored image, analyzing the colored image for its individual colors using the co-ordinates denoting all its 3-dimensional properties of the said image by using an inbuilt software;
   v. denoting the concentrations of the various constituents eluted with time;
   vi. generating a chromatogram based on color analyzed, having peaks at various retention times along with conjugative properties of the molecules;
   vii. identifying the compounds in the said ingredients by the UV-Vis absorptive properties of the various constituents in the image;
   viii. identifying, determining and classifying the compounds eluted as polar, medium polar and less or non-polar based on the polarity and conjugative properties;
   ix. generating a barcode for a selected peak using X axis as Retention Time, Y axis as Wavelength, R as number of Red Pixels, G as number of Green Pixels and B as number of Blue Pixels; and
   x. generating a database of fingerprints and barcodes and identifying the respective compounds in the samples.
2. A method as claimed in claim 1 wherein the solvents with different polarities are selected based on the hydrophilic and hydrophobic nature of the sample under study, ethyl alcohol is used for standardization of medicines.

3. A method as claimed in claim 1 wherein the fingerprints are developed for the same medicine extracted under different pH ranges.

4. A method as claimed in claim 1 wherein the HPLC apparatus used is selected from any commercially available HPLC apparatus with the Photo Diode Array detector, preferably with a gradient or ternary system of pumps.

5. A method as claimed in claim 1 wherein the pH and polarity of the mobile phase is controlled by varying the ratio of the mixture 0 to 100% of an aqueous solvent, water or a buffer at a required pH by using a salt (like Potassium Di-Hydrogen orthophosphate or Di potassium hydrogen orthophosphate and phosphoric acid maintaining the required pH) with a non-aqueous solvent.

6. A method as claimed in claim 1 wherein the non-aqueous, organic and aqueous, water or buffer at a known pH are the solvents used in step 1(iii) and are selected based on the range of polarity.

7. A method as claimed in claim 1 wherein converting the contour chromatograms into a colored image comprising the conjugative and polarity properties of the constituents of the medicine under study.

8. A method as claimed in claim 1wherein, the therapeutic efficacy of a medicine (single or formulated) is assessed using the quality of the constituents present in a particular polarity and UV-Vis absorptive zone.

9. A method as claimed in claim 1 wherein the software generates a barcode for a selected peak or peaks or image using the X axis as Retention Time, Y axis as Wavelength, R as number of Red Pixels, G as number of Green Pixels and B as number of Blue Pixels as the coordinates, provided by the software, which makes the product propriety for an industry.

10. A method as claimed in claim 1 wherein the software used is called Rainbow having the following features:

(a) a software with a facility of opening chromatographic fingerprint images in different Formats (extensions) like .BMP, JPEG, TIF, GIF from the file folders and analyze it for different colors present in the image with single pixel sensitivity;

(b) a software with a facility of display of the pixel information in the form of 1.a graph having a scale of X (0-(min. time scale) and Y (200-800nm) coordinates and 2. a Pie diagram with individual values of each peak (Automatic and Manual) in two separate columns beside the graph;

(c) software with a facility of printing all the data generated after analysis using PRINT Icon;

(d) a software with a facility of changing the page setup for printing using PAGE SETUP Icon;

(e) a software with a facility of selecting a part of the image and analyze using RESIZE Icon;

(f) a software with a facility of opening any number of image analysis windows for different images, and display of status in WINDOW icon;

(g) a software with a facility of dividing the image in to three Zones at 20 min interval, using ZONE icon;

(h) a software with a facility of inverting the selected image using INVERT icon;

(i) a software with a facility of switching over to Notepad, Word pad and MS Word, using EDITOR icon;

(j) a software with a facility of operational information about various features of the Software using the HELP icon; and

(k) software with a facility of saving the data generated using SAVE AS icon as. JEPG file format.

11. A software based data processing of 3 D chromatograms and color contour image of an ingredient, said processing comprising:

a. analyzing (extracting colors) the colored contour image based on the selection of various colors (with standards mentioned in release notes, life cycle,

processing) denoting the concentrations of the various constituents eluted with time, and polarity based on retention time;

b. analyzing the 3-D chromatograms of the medicine using all its 3 dimensional properties of the image;

c. generating a chromatogram having peaks at various retention times along with conjugative properties of the molecules eluted with time in a specified order of polarity;

d. identifying the compounds in the said molecules by the UV-Vis absorptive properties of the various constituents in the image;

e. correlating the reported biological, therapeutic activity of the of various constituents present in the medicines understudy based on the polarity and the conjugative properties of the molecules by dividing the fingerprint into therapeutic zones on X and Y axis;

f. generating a barcode for a selected peak(s) using the image coordinates viz., X for retention time, Y for wavelength, R for number of red pixels, G for number of green pixels and B for number of blue pixels, provided by the proposed software;

g. generating a database of fingerprints and barcodes for the samples, facilitating all kinds of database utilities like Enterprise Resource Planning (ERP) and Customer Resource Management (CRM) applications; and

h. generating a database of the 'display widows' for all the samples to be used by the ENTERPRISE RESOURCE PLANNING (ERP) and CUSTOMER RELATIONSHIP MANAGEMENT (CRM) type of business applications.

12. A method as claimed in claim 11 wherein the solvents used for extraction is selected based on the polarity, hydrophilic and hydrophobic nature of the constituents, sample and its constituents under study.

13. A method as claimed as claimed in claim 11 wherein the HPLC apparatus used is selected from any commercially available HPLC apparatus with the Photo Diode Array detector, preferably with a gradient or ternary system of pumps.

14. A method as claimed in claim 11 wherein, the polarity of the mobile phase of a non-aqueous and an aqueous solvent of a specific pH is controlled by varying the ratio of the mobile phase from 0% to 100% of an aqueous solvents like water or a buffer of a known pH, along with a non-aqueous solvent or vice-versa.

15. A method as claimed in claim 11 wherein, on analysis of 3-D and contour chromatograms using new software called Rainbow, that gives a chromatogram with retention time and wavelength on its X and Y-axis.

16. A method as claimed in claim 11, wherein, on analysis of 3-D and contour chromatograms using new software which gives a data having indicated the vitiation of doshas quantitatively in percentage ratio.

17. A method as claimed in claim 11 wherein, a single solvent ethanol is used for extraction of the constituents; same analytical conditions and instrumental parameters were used for all samples to bring the therapeutic generalizations. The therapeutic standardization is thus achieved.

18. A method as claimed in claim 11 wherein the software used is called Rainbow having the following features:

i. a software with a facility of opening chromatographic fingerprint images in different Formats (extensions) like .BMP, JPEG, TIF, GIF from the file folders and analyze it for different colors present in the image with single pixel sensitivity;

ii. a software with a facility of display of the pixel information in the form of 1.a graph having a scale of X (0-(min. time scale) and Y (200-800nm) coordinates and 2. a Pie diagram with individual values of each peak (Automatic and Manual) in two separate columns beside the graph;

iii. software with a facility of printing all the data generated after analysis using PRINT Icon;

iv. a software with a facility of changing the page setup for printing using PAGE SETUP Icon;

v. a software with a facility of selecting a part of the image and analyze using RESIZE Icon;

vi. a software with a facility of opening any number of image analysis windows for different images, and display of status in WINDOW icon;

vii. a software with a facility of dividing the image in to three Zones at 20 min interval, using ZONE icon;

viii. a software with a facility of inverting the selected image using INVERT icon;

ix. a software with a facility of switching over to Notepad, Word pad and MS Word, using EDITOR icon;

x. a software with a facility of operational information about various features of the Software using, the HELP icon; and

xi. software with a facility of saving the data generated using SAVE AS icon as. JEPG file format.

19. A computational method of chromatographic finger printing, chemical and therapeutic standardization and bar coding of organic and organo-metallic molecules from a plant, animal or a naturally available or man made materials used as medicines, said method comprising

a) selection of medicines and extraction of the constituents,

b) separation of the constituents into individual constituents, generating and converting the 3-D and contour chromatograms into fingerprints,

c) analyzing the fingerprints using the software developed, and

d) interpretation the data.

20. A method as claimed in claim 19 wherein, it provides chemical analysis of the constituents present in the medicine under study and their conjugative and polarity properties indicating the therapeutic efficacy as per the traditional concepts of the medicine using the new software developed.

21. A method as claimed in claim 19 wherein, it provides a novel concept of chromatographic finger printing of herbal medicines which is useful for the quick identification of the actual profile of the compounds present in the medicine under use along with their therapeutic efficacy of the constituents.

22. A method as claimed in claim 19 wherein, it provides a novel chromatographic finger printing of herbal medicines and formulations using the contour and 3-D chromatograms of the herbal medicines and formulations is proposed and they are developed on a Photo Diode Array Detector (PDA) of a High Pressure Liquid Chromatograph. This delineates the data of the spectral properties of the constituents present in the herbal medicines presented in a specific order of polarity under similar experimental analytical conditions.

23. A method as claimed in claim 19 wherein, said method provides UV-Visible spectra of the compounds having displayed the conjugative and polarity properties of the molecules and the concentration of the individual concentrations of the molecules along with the polarity of the molecules.

24. A method as claimed in claim 19 wherein, said method provides UV-VIS spectra of all the constituents shown in a single image "The Chromatographic Fingerprint", the said fingerprint becomes the blue print of the constituents present in an herbal medicine or formulation for an assay and quick identification of the medicine understudy.

25. A method as claimed in claim 24 wherein, same standard analytical parameters like extraction with same solvent ethyl alcohol, same run time 0-60min, same mobile phase acetonitrile along with phosphate buffer having a pH in the range of 5.5-7.5, and a same UV-Visible Range of 200-800nm for fingerprinting and chemical and therapeutic standardization.

26. A method as claimed in claim 24 wherein, fingerprinting is used for the study of adulterated, substituted, contradictual and commercial food and drug samples and to identify the pure and impure.

27. A method as claimed in claim 24 wherein, fingerprinting method is used for identifying the chemical constituents present in it for the purpose of process standardization, quality control activities and therapeutic standardization of Allopathic, Ayurvedic, Homoeo, Siddha, Unani, Chinese, Tibetan, Kampo (Japanese) medicines.

28. A method as claimed in claim 24 wherein, fingerprinting method is used for the study of variation of chemical constituents due to various ecological factors, geological factors, genotypic and phenotypic variations (in plants) in naturally occurring samples and to identify and standardize the chemical constituents in them.

29. A method as claimed in claim 24 wherein, fingerprinting is used for the study of chemical constituents in synthetically prepared samples and to identify and standardize the chemical constituents in them for chemical and therapeutic standardization which ever is applicable.

30. A method as claimed in claim 24 wherein, fingerprinting is used for the study of chemical constituents in herbal products of single medicine samples and to identify the chemical constituents in them for chemical and therapeutic standardization.

31. A method as claimed in claim 24 wherein, fingerprinting is used for the study of chemical constituents in herbal products of formulated medicine samples and to identify the chemical constituents in them for chemical and therapeutic standardization.

32. A method as claimed in claim 24 wherein, fingerprinting is used for the study of variation of chemical constituents in biological samples and to identify and standardize the chemical constituents in them.

33. A method as claimed in claim 24 wherein, fingerprinting is used for the study of variation of chemical constituents in different brands of products of single and formulated food and medicine samples and to identify the chemical constituents in them for chemical and therapeutic standardization.

34. A method as claimed in claim 24 wherein, preparation of a database of a large number samples gives many generalizations of the therapeutic efficacy of a particular group of plants, classified as a group for a particular disease or therapeutic classification.

35. A method as claimed in claim 24 wherein, fingerprinting of medicines facilitates to categorize and quantify the constituents of a medicine based on polarity and

conjugation from 3-D and contour chromatograms and assess the therapeutic efficacy of the medicine on which humors it is going to act (vitiate).

36. A method as claimed in claim 24 wherein, fingerprinting enables to understand and standardize the Physico-Chemical properties of the medicines like color for the use of therapeutic standardization of medicines and humors using conjugative and polarity properties given in the chromatographic fingerprints.

37. A method as claimed in claim 24 wherein, the fingerprinting method enables to understand and standardize the Physico-Chemical properties of the medicines like Tastes (Rasa) like Sour, Salty, Pungent, Bitter, Astringent (Amla, Lavana, Katu, Tikta, Kashaya as described in Ayurveda) used for therapeutic standardization using conjugative and polarity properties shown in the chromatographic fingerprints.

38. A method as claimed in claim 24 wherein, the fingerprinting method enables to understand and standardize the Physico-Chemical properties of the medicines like Property, Potency, Metabolite, Specific properties like Chirality of the molecules (Guna, Veerya Vipaka, Prabhava) used for the therapeutic standardization using conjugative and polarity properties of the individual constituents and the whole medicine shown in the chromatographic fingerprints.

39. A method as claimed in claim 24 wherein, the fingerprinting method enables to understand and standardize the Physico-Chemical properties (Gunas) of the medicines like Cold, Hot, Slow in action, Sharp in action, Heavy, Light, Soft Lubricated Supple, Dry (Sheeta, Ushna, Manda, Teekshna, Guru, Laghu, Snigdha, Rooksha as described in Ayurveda) used for the therapeutic standardization using conjugative and polarity properties of the medicines shown in chromatographic fingerprints.

40. A software based data processor of 3 D chromatograms and color contour image of an ingredient, said processor comprising computing means and capable of:

i) an analyzer (extracting colors) for analyzing the colored contour image based on the selection of various colors (with standards mentioned in

release notes, life cycle, processing) denoting the concentrations of the various constituents eluted with time, and polarity based on retention time;

ii) an analyzer for analyzing the 3-D chromatograms of the medicine using all its 3 dimensional properties of the image;

iii) a means for generating a chromatogram having peaks at various retention times along with conjugative properties of the molecules eluted with time in a specified order of polarity;

iv) an identifier for identifying the compounds in the said molecules by the UV-Vis absorptive properties of the various constituents in the image;

v) a means for correlating the reported biological, therapeutic activity of the of various constituents present in the medicines understudy based on the polarity and the conjugative properties of the molecules by dividing the fingerprint into therapeutic zones on X and Y axis;

vi) a means for generating a barcode for a selected peak(s) using the image coordinates viz., X for retention time, Y for wavelength, R for number of red pixels, G for number of green pixels and B for number of blue pixels, provided by the proposed software;

vii) a means for generating a database of fingerprints and barcodes for the samples, facilitating all kinds of database utilities like Enterprise Resource Planning (ERP) and Customer Resource Management (CRM) applications; and

viii) a means for generating a database of the 'display widows' for all the samples to be used by the ENTERPRISE RESOURCE PLANNING (ERP) and CUSTOMER RELATIONSHIP MANAGEMENT (CRM) type of business applications.

41. A processor as claimed in claim 40 wherein, the solvents used for extraction is selected based on the polarity, hydrophilic and hydrophobic nature of the constituents, sample and its constituents under study.

42. A processor as claimed in claim 40 wherein, the HPLC apparatus used is selected from any commercially available HPLC apparatus with the Photo Diode Array detector, preferably with a gradient or ternary system of pumps.

43. A processor as claimed in claim 40 wherein, the polarity of the mobile phase of a non-aqueous and an aqueous solvent of a specific pH is controlled by varying the ratio of the mobile phase from 0% to 100% of an aqueous solvents like water or a buffer of a known pH, along with a non-aqueous solvent or vice-versa.

44. A processor as claimed in claim 40 wherein, on analysis of 3-D and contour chromatograms using new software entitled " Rainbow " that gives a chromatogram with retention time and wavelength on its X and Y-axis.

45. A processor as claimed in claim 40 wherein, on analysis of 3-D and contour chromatograms using new software which gives a data having indicated the vitiation of doshas quantitatively in percentage ratio.

46. A processor as claimed in claim 40 wherein, a single solvent ethanol is used for extraction of the constituents; same analytical conditions and instrumental parameters were used for all samples to bring the therapeutic generalizations. The therapeutic standardization is thus achieved.

47. A processor as claimed in claim 40 wherein, the software Rainbow has the following features:

(a) It is software entitled '**Rainbow**';

(b) A software with a facility of opening chromatographic fingerprint images in different Formats (extensions) like .BMP, JPEG, TIF, GIF from the file folders and analyze it for different colors present in the image with single pixel sensitivity;

(c) A software with a facility of display of the pixel information in the form of 1.a graph having a scale of X (0-(min. time scale) and Y (200-800nm) coordinates and 2. a Pie diagram with individual values of each peak (Automatic and Manual) in two separate columns beside the graph;

(d) Software with a facility of printing all the data generated after analysis using PRINT Icon.

(e) A software with a facility of changing the page setup for printing using PAGE SETUP Icon;

(f) A software with a facility of selecting a part of the image and analyze using RESIZE Icon;

(g) A software with a facility of opening any number of image analysis windows for different images, and display of status in WINDOW icon;

(h) A software with a facility of dividing the image in to three Zones at 20 min interval, using ZONE icon;

(i) A software with a facility of inverting the selected image using INVERT icon;

(j) A software with a facility of switching over to Notepad, Word pad and MS Word, using EDITOR icon;

(k) A software with a facility of operational information about various features of the Software using, the HELP icon; and

(l) Software with a facility of saving the data generated using SAVE AS icon as JEPG file format.

48. Use of fingerprints of contour and 3 -D chromatograms of the chemical are the basis for identification of chemical constituents to limit the scope of the invention